RAPIDITY OF ACTIVATION IN THE FERTILIZATION OF NEREIS.

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The following experiments were designed as a test of the rapidity of the action of the spermatozoön of *Nereis* in relation to the initiation of the processes of maturation. The almost instantaneous effect of the contact of the spermatozoön in stimulating the formation of the fertilization membrane and, in this case, of the jelly is well known. It is also usually observed that, in the case of those eggs in which maturation follows insemination, that polar-body formation will occur without fail even if later observation shows that no cleavage follows. It might be conceived that the continued action of the spermatozoön were necessary to cause maturation. The results outlined below indicate that maturation in *Nereis* follows almost, if not quite as brief an application of the stimulus as is necessary to initiate the jelly formation.

The experiments of Lillie ('11) on *Nereis limbata* showed clearly that the removal of the spermatozoön later than twenty-one minutes after insemination by a process of centrifuging did not interfere with the maturation of the egg. It was, however, found impossible by this method to remove the sperm earlier than twenty-one minutes after fertilization. Various workers have suggested (I am indebted to Dr. Chambers for first calling this to my attention) that the Barbour apparatus for micro-dissection offered a means of removal during this earlier period. The manipulation of the instrument for this purpose has proved most successful. The spermatozoön may readily be removed shortly after attachment and with more difficulty later because of the increasing strength of adherence to the egg and the viscidity of the head of the spermatozoön. The viscidity is shown in attempts to remove the spermatozoön at about 35 minutes after insemination. The head, remaining attached in the region of the perforatorium may be extended by

the needle some five or ten times its original length. In these later stages the method of removal by centrifuging is more practical.

The experiments were made during the summer of 1919 at the Marine Biological Laboratory at Woods Hole, Mass. Sperm and eggs were obtained from animals caught the evening before and kept in the upper compartment of the refrigerator during the night. Sperm dilutions of about 1/400 to 1/500 were used for most of the experiments. This dilution is not such as will insure that only one spermatozoön will come in contact with each egg (Lillie, '15), but it is sufficiently dilute so that polyspermy rarely results. Polyspermy was noted in a few of the eggs in experiments 6 and 7. The eggs were placed in a dilute suspension of chinese ink, at once fertilized and transferred to the coverslip which forms the roof of the moist chamber. The coverslips had been previously carefully cleaned of all traces of oil or grease in order to allow the drop of water to spread, thus forming a thin film which compresses the eggs slightly as is necessary in order to hold the egg firmly during the operation. The apparatus was always in complete readiness for the operation and it was usually possible to locate promptly an attached spermatozoön and to carefully push or rub it from its point of attachment to the egg. Spermatozoa remained immobile after detachment. Two or three operations were usually carried out on each coverslip. More were not attempted because the delay would allow too great an evaporation of water in the necessarily imperfect moist chamber, thus causing greater compression of the egg and also greater concentration of the sea water. The position of the operated eggs among others was plotted, the coverslip removed and placed under a binocular microscope, the selected eggs were isolated and removed to separate dishes for observation. Frequently the remainder of the eggs on the cover slip was also removed for cleavage counts (Control 2). These were the last to be removed and in some cases had by that time undergone considerable compression (in other cases the cover slip was slightly flooded to prevent this). This may in part account for the low percentage cleavage recorded frequently under Control 2.

The eggs were then kept under observation to note the forma-

tion of the polar bodies and in the second series (Table II.) to note possible cleavage. In all but two of the sixty eggs the polar bodies formed. I am inclined to think that as these two were among the earlier ones observed in this case the negative record may be due to faulty observation. Also in only three cases and one of these from a clearly polyspermic lot of eggs was cleavage observed. The time of removal varied from 1¾ minutes to 13½ minutes after insemination. It is impracticable to remove the sperm in most cases earlier than two minutes after insemination as it is difficult to discriminate between spermatozoa lying against the egg and those that have effected an attachment. The average of elapsed time from insemination to removal was 6.2 minutes and in sixteen cases the spermatozoön was removed in less than 4 minutes after attachment, and in five cases in less than 3 minutes. It seems therefore clear that in so far as maturation is concerned, the full stimulating effect of the spermatozoön is effective within a very few minutes after attachment and quite possibly it is only a matter of seconds.

The results clearly support the concept that the first phase of activation of the egg-membrane formation and maturation is initiated by the spermatozoön which "activates a substance, or ferment-like bodies, contained within the egg" (Lillie, '19, p. 159), rather than by the continuous introduction of some lysin-like substance through the slender perforatorium. For it seems improbable that the spermatozoön could introduce in less than two minutes through the perforatorium which has a cross section area of perhaps one ten-millionth of the surface area of the egg, a sufficient amount of material to take part in reactions throughout the egg. It should, however, be noted that under certain conditions (heating, Just, '15) it is possible to initiate jelly formation without maturation following. This may be taken to indicate that a lesser stimulus gives a lesser result and possibly if it were practicable to remove the sperm more promptly that jelly formation only would result. On the other hand in the cited case we may be dealing with a stimulus differing in kind rather than in degree. It should also be noted that these results are not at variance with the concept that cortical changes form an all important intermediate step in the activation of the egg.

In some cases (Table II.) it was possible to note the time of formation of the first polar body. The average of elapsed time after insemination was 35.8 minutes and no correlation was noted between the duration of attachment of the sperm and the time of formation of the polar bodies.

TABLE I.

Date.	Experiment Number.	Time of Fertilization.	Egg.	Time of Removal of Sperm.	Minutes Elapsed.	Polar Body Formation.	Cleavage.	Controls.	
								No. 1.	No. 2.
July									
17	1	10.48	A	10.56	8	+	−	+	60%
			B	11.02	14	+	−	+	"
	2	2.44	A	2.54	10	+	−[1]	+	90%
			B	2.58	14	+	−[1]	+	"
18	3	5.29	A	5.32½	3½	−	−[1]	+	65%
			B	5.37½	8½	+	−[1]	+	"
19	4	9.59	A	10.03	4	−	−[1]	+	70%
			B	10.08½	9½	+	−[1]	+	"
			C	10.10	11	+	−[1]	+	"
20	5	2.29½	A	2.33½	4	+	−	+	60%
	6	2.40	B	2.51	11	+	−[2]	+	"
21	7	9.40½	A	9.48	7½	+	+[2]	+	90%
	8	10.14½	A	10.18½	4	+	+	+	40%
			B	10.21¾	7¼	+	+	+	"
	9	11.26	A	11.27¾	1¾	+	−[1]	+	50%
			B	11.32	6	+	−[1]	+	"
	10	2.54½	A	3.00	5½	+	−	+	70%
			B	3.03	8½	+	−	+	"
	11	3.13	A	3.16½	3½	+	−[1]	+	
			B	3.21½	8½	+	−[1]	+	

As noted in Table I. some eggs were fixed at about time of the first cleavage with view to study of cytological changes. Too few of these have survived the ordeal of embedding and sectioning to make any detailed study valuable. I noted that in some cases only chromosomal vesicles and in other cases well-formed chromosomes were present at the time of the first cleavage spindle. This may be compared with the observation of Lillie ('15) where chromosomes were formed in eggs from which sperm were removed by centrifuging but not in eggs which were caused to maturate by centrifuging without insemination.

The appended tables outline the experiments. Full data are

[1] Egg fixed at about time of expected first cleavage. No indication of cleavage at that time.

[2] Poly-spermy observed in same lot on cover-slip.

not present in all cases. The experiments were in part preliminary and the desirability of various controls and observation became apparent as they progressed. In as much as the work

TABLE II.

Date.	Experiment Number.	Time of Fertilization.	Egg.	Time of Removal of Sperm.	Minutes Elapsed.	Polar Body Formation.	Time Formed.	Minutes Elapsed.	Cleavage.	Controls.		
										No. 1.	No. 2.	No. 3.
July												
23	12	9.47	A	9.50	3	+	10.19	33	−	+	60%	
			B	9.53½	6½	[3]				+		
			C	9.54½	7½	+	10.20	34	−	+	"	
	13	10.20	A	10.23¼	3¼	+	10.52	32	−	+	80%	
			B	10.25⅓	5⅓	+			−	+	"	
	14	11.05½	A	11.08½	3	+			−	+		
	15	11.36½	A	11.38½	2	+			−	+		
			B	11.42½	6	+			−	+		
			C	11.43½	7	+			−	+		
	16	2.40	A	2.48½	8½	+	3.21	31	−	+	90%	
			B	2.53½	13½	+	3.17	37	−	+	"	
	17	3.16½	A	3.24½	8	no. obs.			−[4]	+		
			B	3.24½	9	+	3.49½	33	−[4]	+		
			C	3.26½	10	+			−[4]	+		
			D	3.28	11½	+			−[4]	+		
24	18	10.15	A	10.18½	3½	+	10.46	31	−[4]	+		
			B	10.22	7	+	10.49	34	−[4]	+		
	19	10.51½	A	10.55	3½	+	11.22½	31	−[4]	+		
			B	10.57½	6	+			−[4]	+		
			C	10.58½	7	no obs.			−[4]	+		
	20	11.26	A	11.29	3	+	11.56½	30½	−[4]	+		
			B	11.33½	6½	+	11.57½	31½	−[4]	+		
	21	3.18½	A	3.21¾	3¼	+	3.53	35	−[4]	+		
			B	3.24	5½	+	3.53	35	−[4]	+		
			C	3.25	6½	+	3.52	34	−[4]	+		
25	22	9.33	A	9.36	3	+	10.05½	32½	−	+	60%	
			B	9.38	5	+	10.06	33	−	+	"	
			C	9.39	6	+	10.06	33	−	+	"	
	23	10.16	A	10.18½	2½	+			−	+	58%	
			B	10.21½	5½	+			−	+	"	
	24	10.48½	A	10.51½	3	+			−	+	57%	
			B	10.52	3½	+			−	+	"	
			C	10.55	6½	+			−	+	"	
			D	10.57	8½	+			−	+	"	
	25	11.34	A	11.37½	3½	+	12.15	41	−	+	45%	
			B	11.43½	9½	+			−	+	"	
	26	2.19¾	A	2.42½	4¾	+	2.57	37¼	−	+	25%	
	27	2.47	A	2.49	2	+	3.25	38	−	+	55%	+
	28	3.54½	A	3.57	2½	+	3.37	42½	−	+	80%	+
			B	4.00	5½	+	3.36	41½	−	+	80%	+
26	29	9.52	A	9.55	3	+	10.27	35	−	+	66%	+
	30	10.29	A	10.32¾	3¾	+	11.02	33	−	+	"	+
			B	10.35	6	+	11.03	34	−	+	57%	
	31	11.05½	A	11.07½	2½	+	11.38½	33	−	+	75%	+
			B	11.10¾	5¼	+	11.42½	37	−	+	"	+

[3] Egg disintegrated.

[4] No specific record in original notes of the absence of cleavage.

was stopped by the end of the *Nereis* "Run" and as the observations in regard to maturation were complete it seems to me to be best to present the work in its present form.

TABLES.

The headings are mostly self-explanatory. Under Polar Body Formation and Cleavage a plus sign (+) indicates that the process was observed and a minus (−) the reverse. Control No. 1 are eggs from the same lots as in the experiments fertilized a few minutes before the experiment began from the sperm of the same male used. Control 2 are eggs not operated on from the coverslip and Control 3 are eggs of the same inseminated lot mixed with chinese ink from which those placed on the cover slip were taken. The plus sign (+) under Controls 1 and 3 indicates the practically 100 per cent. cleavage usually realized with *Nereis* eggs.

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